BIOCHEMICAL ANALYSIS OF FLOWERS OF *Vinca major,* A MEDICINAL WEED PLANT OF HILLY AREAS OF PAKISTAN

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DOI: https://doi.org/10.28941/pjwsr.v27i4.1014

ABSTRACT

Vinca major L. is an evergreen perennial weed of family Apocynaceae, growing naturally in hilly areas of Hazara region of Khyber Pakhtunkhwa, Pakistan. Flowers of this weed were collected during June 2021, shade dried and extracted in pure methanol for two weeks. After filtration, the extract was analyzed by GC-MS for the identification of possible bioactive compounds. α-Amyrin was the major compound (32.49%) followed by lup-20(29)-en-3-ol, acetate, (3β)- (25.72%). Moderately abundant compounds included γ-sitosterol (8.78%), β-amyrone (7.25%), cyclohexane, 1,3,5-triphenyl- (7.01%), olean-12-en-3-ol, acetate, (3β)- (5.68%), and *n*-hexadecanoic acid (3.18%). Some of the identified compounds have various important biological properties including anti-inflammatory, antimicrobial, anticancer, antimalarial, antioxidant, antidiabetic, antitumor, etc.

Keywords: Bioactive compounds, Flower extract, GC-MS analysis, Khyber Pakhtunkhwa, *Vinca major*.

Citation: Javaid, A.; M.F.H. Ferdosi; I.H. Khan; A. Shoaib; H.M. Saeed; M.A. Hassan. 2021. Biochemical Analysis of Flowers of *Vinca major*, A Medicinal Weed Plant of Hilly Areas of Pakistan. Pak. J. Weed Sci. Res., 27(4): 537-546.

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INTRODUCTION

Indigenous medicinal plants represent a reservoir of natural products that have been used by human beings since ancient times. This has resulted in the use of large number of plants with curative properties against several diseases in many parts of the world (Dar *et al.,* 2017; Jamshidi-Kia *et al.,* 2018; Khan and Javaid, 2020, 2021). Cost and side effects of conventional medications are the major factors responsible for revival of herbal medicines (Uritu *et al.,* 2018). Recent literature also shows the prevalent use of herbs as medicines across the globe (Saravanan *et al.,* 2018; Khan and Javaid, 2019). So far, a lot of important drugs are derived from traditional medicinal herbs (Manandhar *et al.,* 2019; Salmerón-Manzano *et al.,* 2020). A variety of compounds comprised of alkaloids, tannins, steroids, saponins, phenolic acids, terpenoids and quinones have been identified, which possess cardioprotective, anticancer, antioxidant, antimicrobial, anti-insect and a variety of other properties (Tungmunnithum *et al.,* 2018).

Pakistan is enriched in medicinal herbs because of varied climatic conditions (Ullah, 2017; Javed *et al.,* 2021). Many of these medicinal plants have been explored for their antifungal, antibacterial, herbicidal and other bioactive properties (Javaid *et al.,* 2020, 2021a; Banaras *et al.,* 2021; Ferdosi *et al.,* 2021a). These are scattered over a large area and about 600 species known well in Pakistan for their medicinal values (Shinwari, 2010). *Vinca major* of family Apocynaceae, is an evergreen perennial weed plant that has medicinal and ornamental values (Arora *et al.,* 2010). It grows well in full sun as well as in shade with a height of up to 25 cm while spreading indefinitely. Phytochemical screening of *V. major* revealed the presence of saponins, alkaloids, phenols, organic acids and sterols exhibiting antioxidant, antimicrobial, antidiabetic and hypotensive properties (Singh *et al.,* 2014; Wei and Liu, 2021). *Vinca* extracts have diverse compounds that act as antineoplastic agents and are also used to treat choriocarcinoma, lymphomas and hodgkin's diseases (Vishwakarma and Prajapati, 2019; González-Burgos and Gómez-Serranillos, 2021). Moreover, aerial plant parts are used traditionally to treat diarrhea, piles, leukemia, malaria, diabetes, sore throat, cough and diabetes (Rajput *et al.,* 2011; Ajaib *et al.,* 2014; Comfort *et al.,* 2019). However, studies regarding phytochemical analysis of flowers of this plant from Pakistan are scarce. Thus, the present study was conducted to identify phytoconstituents of *V. major* flowers through GC-MS analysis and to document its medicinal importance.

MATERIALS AND METHODS

Disease free and mature flowers of *V. major* were collected from Murree, Pakistan. Plucked flowers were packed in plastic bags and shifted to laboratory for further analysis. Flowers were washed in water and completely dried at 35 ºC in a hot air oven. After evaporating moisture from flower, the dried flowers were then finely crushed into a powdered form with pestle and mortar. Ten grams of this material were soaked in 50 mL of analytical graded methanol and kept for two weeks so that maximum bioactive compounds can be extracted. Thereafter, the extract was filtered through a filter paper. Following filtration, 2 mm extract was collected in a 5 mL vial for GC-MS analysis.

Fig. 1: *Vinca major* growing in Murree, Pakistan.

Various biologically active compounds were identified from methanolic flower extract using GC-MS analysis as described by Ferdosi *et al.* (2020). Gas chromatography (GC) was performed on 7890B Model Machine, Agilent Technologies (USA) with Column DB-5ms having dimensions of 30 m × 0.25 µm × 0.25 µm. Helium, an inert gas (having 4 amu atomic mass) was used as a carrier gas. Injection volume was 1 µL, while oven initial temperature was 80 ºC that was raised to 10 ºC min^{-1} up to 300 ºC. MS analysis was performed on 5977A Model Machine, Agilent Technologies (USA) with scan range of 50–500 m/z; solvent delay time was 5 min. Source temperature was 230 ºC with 50 min run time. The resulted spectrum was then analyzed with NIST library of 2017 version for the phytochemical characterization and the compounds were arranged in the ascending order of their retention times. Relative abundance of the compounds was analyzed using of peaks heights in the chromatogram. Chemical structures of various compounds in the extract were drawn using ChemDraw software.

In order to collect information on biological activities of the identified compounds, different databases including Science Direct, PubMed, SciELO, Google Scholar, Directory of Open Access Journals and Crossref were surveyed.

RESULTS AND DISCUSSION

Fourteen compounds were detected in GC-MS analysis of methanolic flower extract of *V. major* (Fig. 2). Details of the identified compounds are presented in Table 1. The most abundant compound was α-amyrin (32.49%) followed by lup-20(29)-en-3-ol, acetate, (3β)- (25.72%). Four compounds namely γ-sitosterol (8.78%), β-amyrone (7.25%), cyclohexane, 1,3,5-triphenyl- (7.01%), olean-12-en-3-ol, acetate, (3β)- (5.68%) and *n*-hexadecanoic acid (3.18%) were ranked as moderately abundant compounds. Compounds such as campesterol (2.11%), cyclododecane (1.17%), pentadecanoic acid, 14-methyl-, methyl ester (1.32%), heneicosane (1.31%), 2-ethylacridine (1.23%), 1,2-bis(trimethylsilyl)benzene (1.42%), were categorized as less abundant ones. Structures of major compounds are shown in Fig. 3.

The principal compound in the flower extract was α-amyrin. Earlier, this compound has been reported in *Cirsium arvense* (Ferdosi *et al.,* 2021b), *Myrcianthes pungens* (Cardoso *et al.,* 2020) and *Strobilanthes callosus* (Singh *et al.,* 2002). It is known for its antimicrobial, antioxidant and anti-inflammatory properties (Singh *et al.,* 2002). In addition, it also showed inhibitory effects against human oxidosqualene cyclase (Chen *et al.,* 2017). Its pharmacological activity is also known in gastrointestinal tract and immunological system (Nogueira *et al.,* 2019), and in the treatment of gingivitis and periodontitis (Pinto *et al.,* 2008). Likewise, β-amyrone isolated from oil-

resins of *Protium paniculatum* also showed anti-inflammatory activity (de Almeida *et al.,* 2015).

The second most abundant compound lup-20(29)-en-3-ol, acetate, (3β)-, also known as lupeyl acetate and lupeol acetate, belongs to triterpenoids group. This compound was isolated from bark of *Artocarpus integra* and showed anticancer activity against breast cancer cells MCF-7 (Suwito *et al.,* 2016). It also possesses numerous other biological activities including anti-inflammatory, antituberculosis, antimalarial, antimicrobial and antinociceptive (Prachayasittikul *et al.,* 2010; Chen *et al.,* 2012), An isomer of this compound, olean-12-en-3-ol, acetate, (3β)- also known as β-amyrin 3- acetate, was found as a moderately occurring compound in this study. It possesses anti-inflammatory potential and also inhibits growth of *Staphylococcus aureus* (Hichri *et al.,* 2003; Akihisa *et al.,* 2010). It has been found as a major constituent in flowers of *C. arvense* and (Fernandes *et al.,* 2013; Ferdosi *et al.,* 2021b).

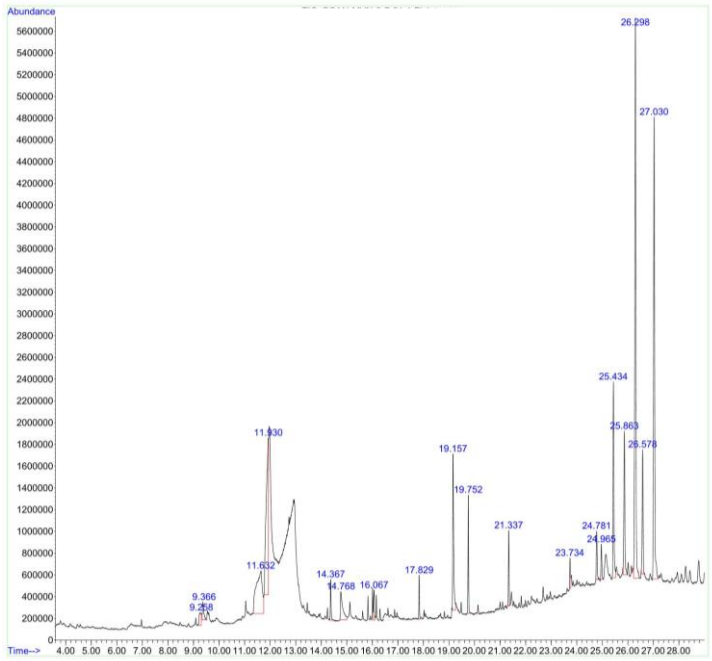

Fig. 1: GC-MS chromatogram of methanolic flower extract of *Vinca major*.

Table 1: Compounds identified in methanolic flower extract of *Vinca major* through GC-MS analysis.

Sr. No.	Names of compounds	Molecular formula	Molecular weight	Retention time (min)	Peak area (%)
1	5-Dodecene, (E)-	$C_{12}H_{24}$	168.31	9.366	1.17
2	Pentadecanoic acid, 14-methyl-, methyl ester	$C_{17}H_{34}O_2$	270.45	14.367	1.32
3	*n*-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.42	14.768	3.18
4	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	292.45	16.067	1.25
5	Heneicosane	$C_{21}H_{44}$	296.57	17.829	1.31
6	Cyclohexane, 1,3,5-triphenyl-	$C_{24}H_{24}$	312.44	19.157	7.01
7	2-Ethylacridine	$C_{15}H_{13}N$	207.27	23.734	1.23
8	Campesterol	$C_{28}H_{48}O$	400.68	24.781	2.11
9	1,2-Bis(trimethylsilyl)	$C_{12}H_{22}Si_2$	222.47	24.965	1.42

	benzene				
10	γ-Sitosterol	$C_{29}H_{50}O$	414.70	25.434	8.78
11	β-Amyrone	$C_{30}H_{48}O$	424.70	25.863	7.25
12	α-Amyrin	$C_{30}H_{50}O$	426.71	26.298	32.49
13	Olean-12-en-3-ol, acetate, (3β)-	$C_{32}H_{52}O_2$	468.75	26.578	5.68
14	Lup-20(29)-en-3-ol, acetate, (3β)-	$C_{32}H_{52}O_2$	468.75	27.030	25.72

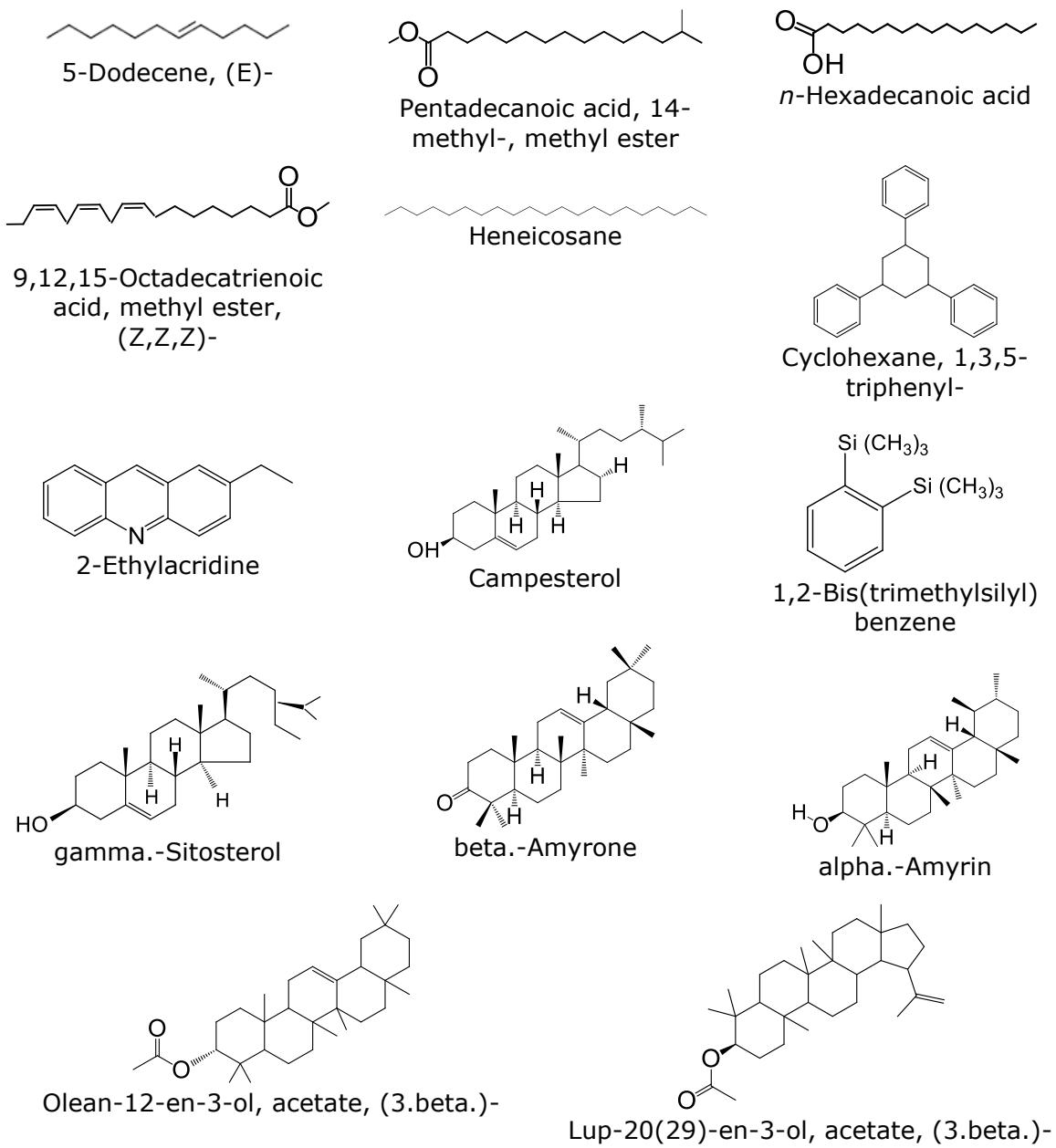

Fig. 3: Structures of compounds present in flower extract of *Vinca major.*

Plant species such as *Lippia nodiflora* and *Acacia nilotica*, with anticancer (Sundarraj *et al.,* 2012) and antidiabetic properties (Balamurugan *et al.,* 2011). 9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)- and pentadecanoic acid, 14-methyl-, methyl ester are fatty acid methyl esters, which have been found in numerous plant species such as *Cannabis sativa,*

Ageratum conyzoides and *Coronopus didymus* (Banaras *et al.,* 2021; Javaid *et al.,* 2018, 2021b). Such compounds showed antifungal and antibacterial activities (Johnson *et al.,* 2011; Bashir *et al.,* 2012). *n*-Hexadecanoic acid, also known as palmitic acid, is a highly biologically active compound, found in many plants (Javaid *et al.,* 2018; Naqvi *et al.,* 2020). It possesses anti-inflammatory, antimicrobial, mosquito larvicidal, hypocholesterolemic, antioxidant and pesticidal properties (Rahuman *et al.,* 2000; Kumar *et al.,* 2010; Aparna *et al.,* 2012; Abubakar and Majinda, 2016). Heneicosane, an alkane, isolated from *Plumbago zeylanica* showed antimicrobial activity against *Streptococcus pneumoniae* and *Aspergillus fumigatus* (Vanitha *et al.,* 2020). Likewise, 2-ethylacridine also possesses antimicrobial and antitumor activities (Vijayakumari and Raj, 2019). Campesterol is a sterol found in plant and cholesterol lowering and anticarcinogenic properties (Choi *et al.,* 2007).

Table 2: Bioactivity of components of methanolic flower extract of *Vinca major*.

Sr. No.	Names of compounds	Bioactivity	Reference
1	5-Dodecene, (E)-	-	-
2	Pentadecanoic acid, 14-methyl-, methyl ester	Antifungal, Antimicrobial	Bashir *et al.* (2012)
3	*n*-Hexadecanoic acid	Antioxidant, pesticidal, anti-inflammatory, mosquito larvicide, hemolytic	Kumar *et al.* (2010); Aparna *et al.* (2012); Abubakar and Majinda (2016)
4	9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	Antibacterial, antioxidant, anticancer, antipyretic, cardioprotective, antiarthritic neural function, Antiandrogenic,	Johnson *et al.* (2011); Akpuaka *et al.* (2013)
5	Heneicosane	Antimicrobial	Vanitha *et al.* (2020)
6	Cyclohexane, 1,3,5-triphenyl-	-	-
7	2-Ethylacridine	Antimicrobial, antitumor	Vijayakumari and Raj (2019)
8	Campesterol	Anti-cholesterol, anticarcinogenic	Choi *et al.* (2007)
9	1,2-Bis(trimethylsilyl) benzene	-	-
10	γ-Sitosterol	Anticancer, antidiabetic	Balamurugan *et al.* (2011); Sundarraj *et al.* (2012)
11	β-Amyrone	Anti-inflammatory	de Almeida *et al.* (2015)
12	α-Amyrin	Antimicrobial Anti-inflammatory, antioxidant, inhibitor of human oxidosqualene cyclase	Singh *et al.* (2002); Chen *et al.* (2017); Cardoso *et al.* (2020)
13	Olean-12-en-3-ol, acetate, (3β)-	Antibacterial, anti-inflammatory	Hichri *et al.* (2003); Akihisa *et al.,* (2010)
14	Lup-20(29)-en-3-ol,	Anticancer, anti-	Prachayasittikul *et al.*

acetate, (3β)-	inflammatory, antituberculosis, antimalarial, antimicrobial, antinociceptive	(2010); Chen *et al.* (2012); Suwito *et al.* (2016)

Conclusion

Flower of *V. major* is a rich source of bioactive substances especially *n*-hexadecanoic acid; α-amyrin, lupeol acetate, γ-sitosterol; heneicosane and fatty acid methyl esters with a number of biological activities including anticancer and antidiabetic.

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